IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Gee *et al.*	Examiner: Bruck Kifle
Serial No.: 10/634,336	Group Art Unit: 1624
Filed: August 4, 2003	Docket No. MP 0070.1CIP
For: **Crown Ether Derivatives**	**MARKED-UP VERSION OF THE CLAIMS**

Commissioner for Patents
U.S. Patent and Trademark Office
Washington, D.C. 20231

Dear Sir:

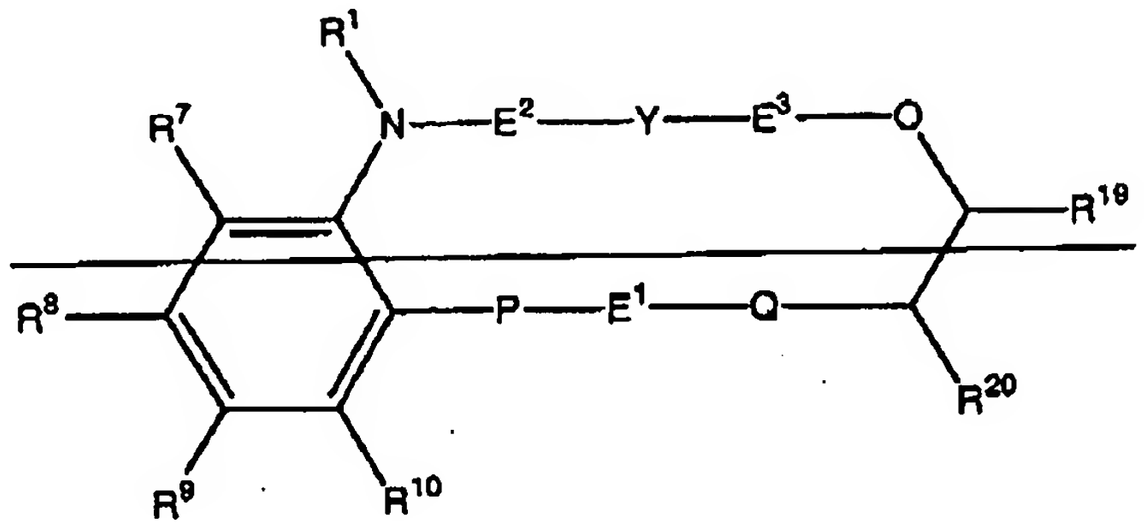
This is in response to the Office Action mailed on January 5, 2006 and is submitted on or before the three month due date of April 5, 2006.

The Examiner is respectfully requested to enter the amendments and examine the claims on the merits.

CERTIFICATE OF TRANSMISSION

I HEREBY CERTIFY THAT THIS PAPER AND THE DOCUMENTS REFERRED AS BEING ATTACHED OR ENCLOSED HEREWITH ARE BEING FACSIMILE TRANSMITTED TO THE UNITED STATES PATENT AND TRADMARK OFFICE ON 4-5-06 TO 1.571.273.8300 By Karen Anderson

1. (Currently Amended) A crown ether chelating compound having formula:



~~wherein~~

~~Y is O, S, NR^4 or is absent, wherein R^4 is selected from the group consisting of H, -L-R_x, -L-S_c, -L-DYE, C_1-C_{18} alkyl, aryl and heteroaryl ring system, which alkyl or ring system is optionally substituted by halogen, azido, nitro, nitroso, amino, C_1-C_6 alkylamino, C_2-C_{12} dialkylamino, cyano, -L-R_x, -L-S_c, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy that is itself optionally substituted by halogen, amino, hydroxy, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, or $-(C=O)-NR^{17}R^{18}$; wherein~~

~~R^{15} is selected from the group consisting of H, C_1-C_6 alkyl, -L-R_x, -L-S_c and -L-DYE;~~

~~R^{16} is selected from the group consisting of H, C_1-C_6 alkyl, benzyl, a biologically compatible esterifying group, a biologically compatible salt, -L-R_x, -L-S_c and -L-DYE;~~

~~R^{17} and R^{18} are independently selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, alpha-acyloxyalkyl, trialkylsilyl, a biologically compatible salt, -L-R_x, -L-S_c and -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;~~

~~each L is independently a covalent linkage;~~

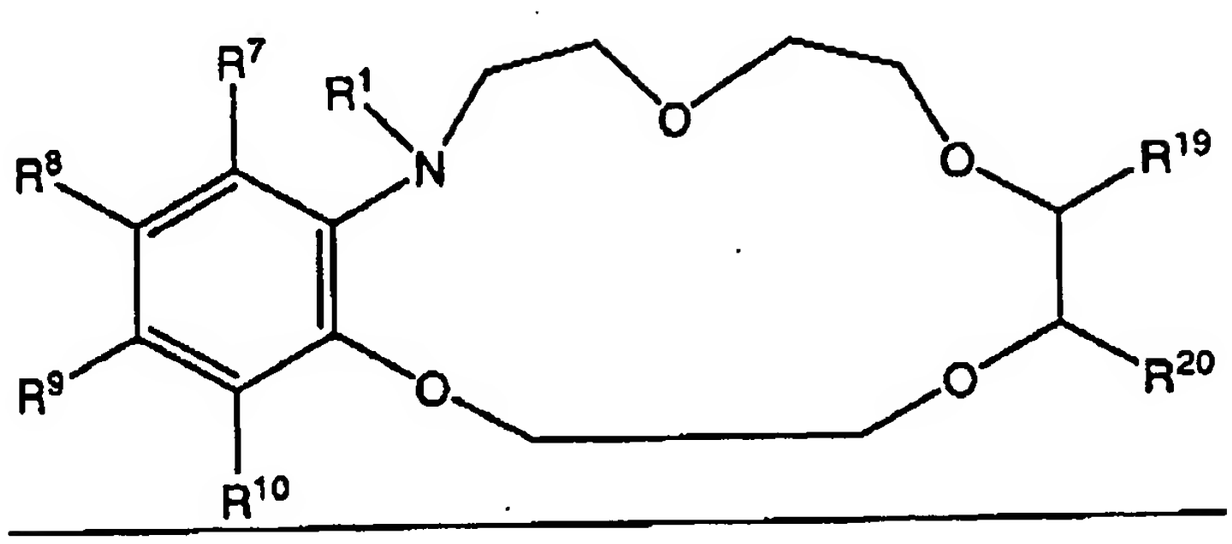
~~each R_x is independently a reactive group;~~

~~each S_C is independently a conjugated substance;~~

~~each DYE is independently a reporter molecule;~~

~~P and Q are independently O, S or NR^3, wherein each R^3 is independently H or C_1-C_6 alkyl;~~

~~E^1, E^2, and E^3 are independently $-(CR^5_2)_n$-, $-(C(O)CH_2)_n$-, $-(CR^5_2)_nO(CR^5_2)_n$- or E^3 is absent, where n = 2, 3 or 4, and each R^5 is independently H or CH_3, or two R^5 moieties on adjacent carbons of one or more of E^1, E^2 or E^3, when taken in combination, form a 5- or 6-membered aliphatic ring;~~



wherein

R^1 is selected from the group consisting of -L-R_X, -L-S_C, -L-DYE, C_1-C_{18} alkyl and C_7-C_{18} arylalkyl, each of which is optionally substituted by halogen, azido, nitro, nitroso, amino, hydroxy, cyano, C_1-C_6 alkoxy, an aryl or heteroaryl ring system, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{15}, -(C=O)-$NR^{17}R^{18}$, C_1-C_6 alkylamino, C_2-C_{12} dialkylamino, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino (-$NR^{17}R^{18}$), hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{15} or -(C=O)-$NR^{17}R^{18}$;

R^{15} is selected from the group consisting of H, C_1-C_6 alkyl, -L-R_X, -L-S_C and -L-DYE;

R^{16} is selected from the group consisting of H, C_1-C_8 alkyl, benzyl, a biologically compatible esterifying group, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE;

R^{17} and R^{18} are independently selected from the group consisting of H, C_1-C_8 alkyl, C_1-C_8 carboxyalkyl, alpha-acyloxyalkyl, trialkylsilyl, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;

each L is independently a covalent linkage;

each DYE is independently a reporter molecule;

each Rx is independently an acrylamide, an activated ester of a carboxylic acid, a carboxylic ester, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an anhydride, an aniline, an amine, an aryl halide, an azide, an aziridine, a boronate, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a silyl halide, a sulfonyl halide, a thiol and a photoactivatable group;

each Sc is independently an amino acid, a peptide, a protein, a polysaccharide, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell or a virus;

R^{19} and R^{20} are independently selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl and C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{15}, or -(C=O)-$NR^{17}R^{18}$;

or R^{19} and R^{20} taken in combination form a fused six-membered benzo moiety that is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by

halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

R^7, R^8, R^9 and R^{10} are independently selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{10}, taken in combination, form a fused six-membered benzo moiety, which is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is optionally substituted by halogen, amino, hydroxy, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{10}, or R^{19} and R^{20}, taken in combination with each other, form a fused DYE.

2. (Canceled)

3. (Canceled)

4. (Currently Amended) The compound according to ~~Claim 3~~ Claim 1, wherein at least one of said R^1, R^7, R^8, R^9, R^{10}, R^{19} or R^{20} is -L-Rx, -L-Sc or -L-DYE or R^8 in combination with R^9 form a fused DYE.

5. (Currently Amended) The compound according to ~~Claim 4~~ Claim 1, wherein said L is a single covalent bond, or a covalent linkage that is linear or branched, cyclic or heterocyclic, saturated or unsaturated, having 1-20 nonhydrogen atoms selected from the group consisting of C, N, P, O and S; and are composed of any combination of ether, thioether, amine, ester, carboxamide, sulfonamide, hydrazide bonds and aromatic or heteroaromatic bonds.

6. (Canceled)

7. (Currently Amended) The compound according to ~~Claim 6~~ Claim 1, wherein said –Rx is selected from the group consisting of carboxylic acid, succinimidyl ester of a carboxylic acid, hydrazide, amine and a maleimide.

8. (Canceled)

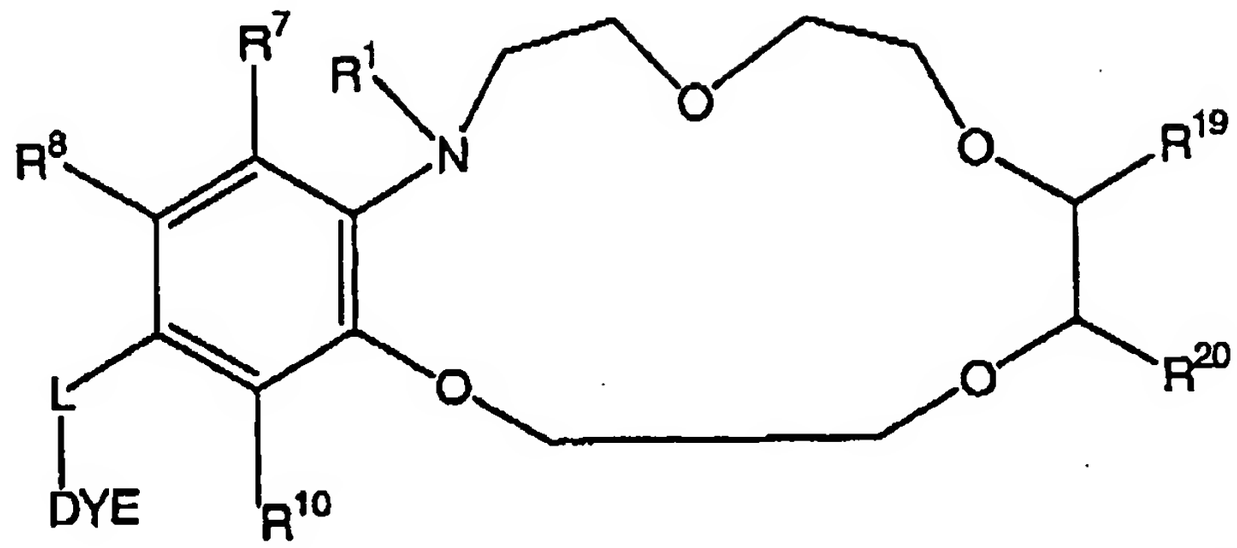
9. (Currently Amended) The compound according to ~~Claim 8~~ Claim 1, wherein said –Sc is selected from the group consisting of an antibody or fragment thereof, an avidin or streptavidin, a biotin, a blood component protein, a dextran, an enzyme, an enzyme inhibitor, a hormone, an IgG binding protein, a fluorescent protein, a growth factor, a lectin, a lipopolysaccharide, a microorganism, a metal binding protein, a metal chelating moiety, a non-biological microparticle, a peptide toxin, a phosphotidylserine-binding protein, a structural protein, a small-molecule drug, or a tyramide.

10. (Currently Amended) The compound according to ~~Claim 5~~ Claim 1, wherein said –DYE is selected from the group consisting of xanthene, borapolyazaindacene, carbocyanine, benzofuran, quinazolinone, indole, a benzazole, oxazine, and coumarin.

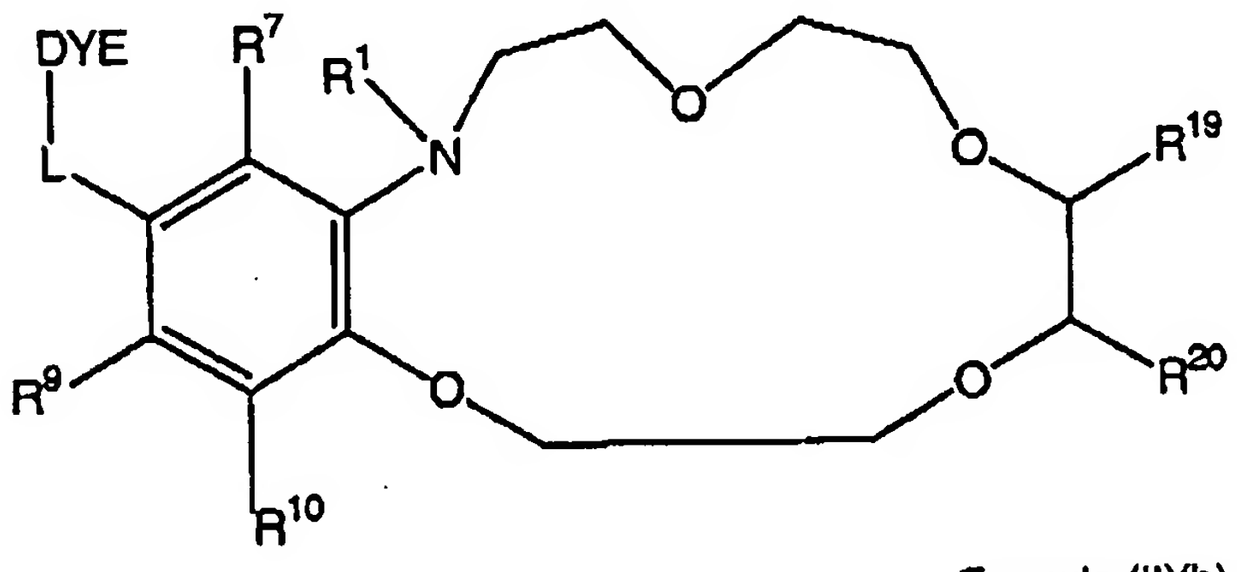
11. (Original) The compound according to Claim 10, wherein said –DYE moiety is independently substituted by a lipophilic group.

12. (Original) The compound according to Claim 11, wherein said lipophilic group is an AM or acetate ester.

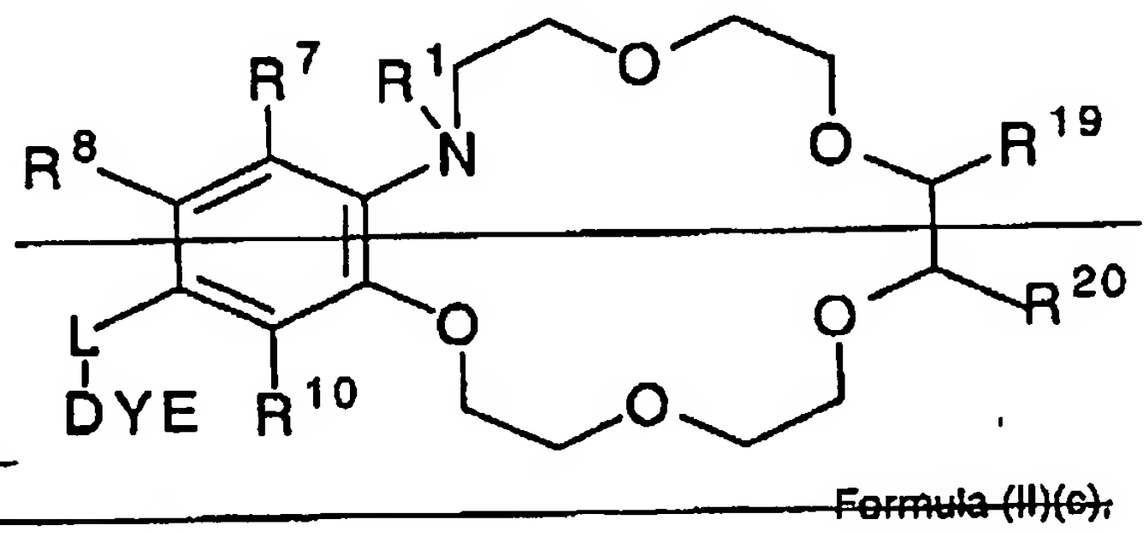
13. (Currently Amended) The compound according to Claim 1, wherein said compound is selected from the group consisting of



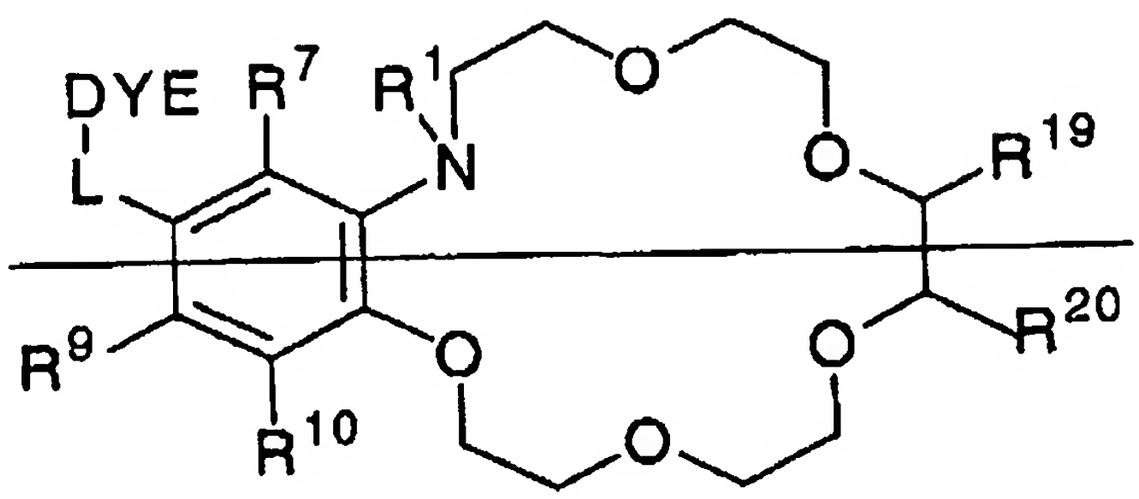
Formula (II)(a), and



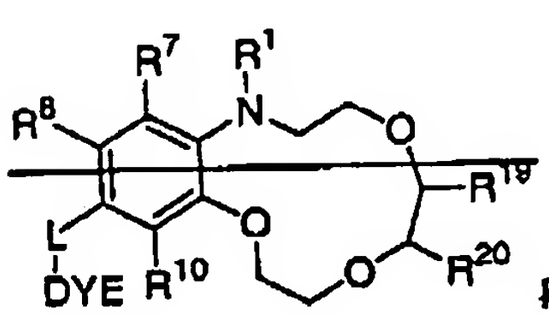
Formula (II)(b). [[,]]



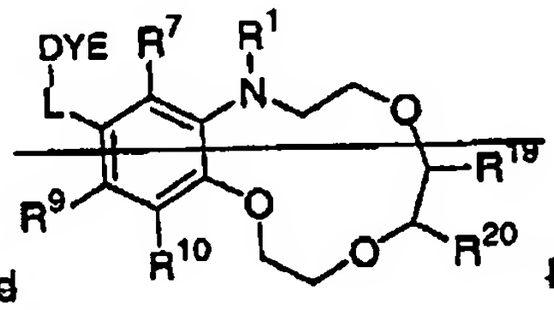
~~Formula (II)(c).~~



~~Formula (II)(d),~~



~~Formula (II)(e) and~~



~~Formula (II)(f).~~

14. (Original) The compound according to Claim 13, wherein said DYE is selected from the group consisting of borapolyazaindacene, xanthene and indole.

15. (Original) The compound according to Claim 13, wherein said DYE moiety is independently substituted by a lipophilic group.

16. (Original) The compound according to Claim 15, wherein said lipophilic group is an AM or acetate ester.

17. (Original) The compound according to Claim 13, wherein R^7, R^8, R^9, R^{10}, R^{19} and R^{20}, when present, are H.

18. (Original) The compound according to Claim 17, wherein R^1 is C_1-C_6 alkyl that is substituted one or more times by amino (-$NR^{17}R^{18}$), –(C=O)-O-R^{16} or –(C=O)-$NR^{17}R^{18}$.

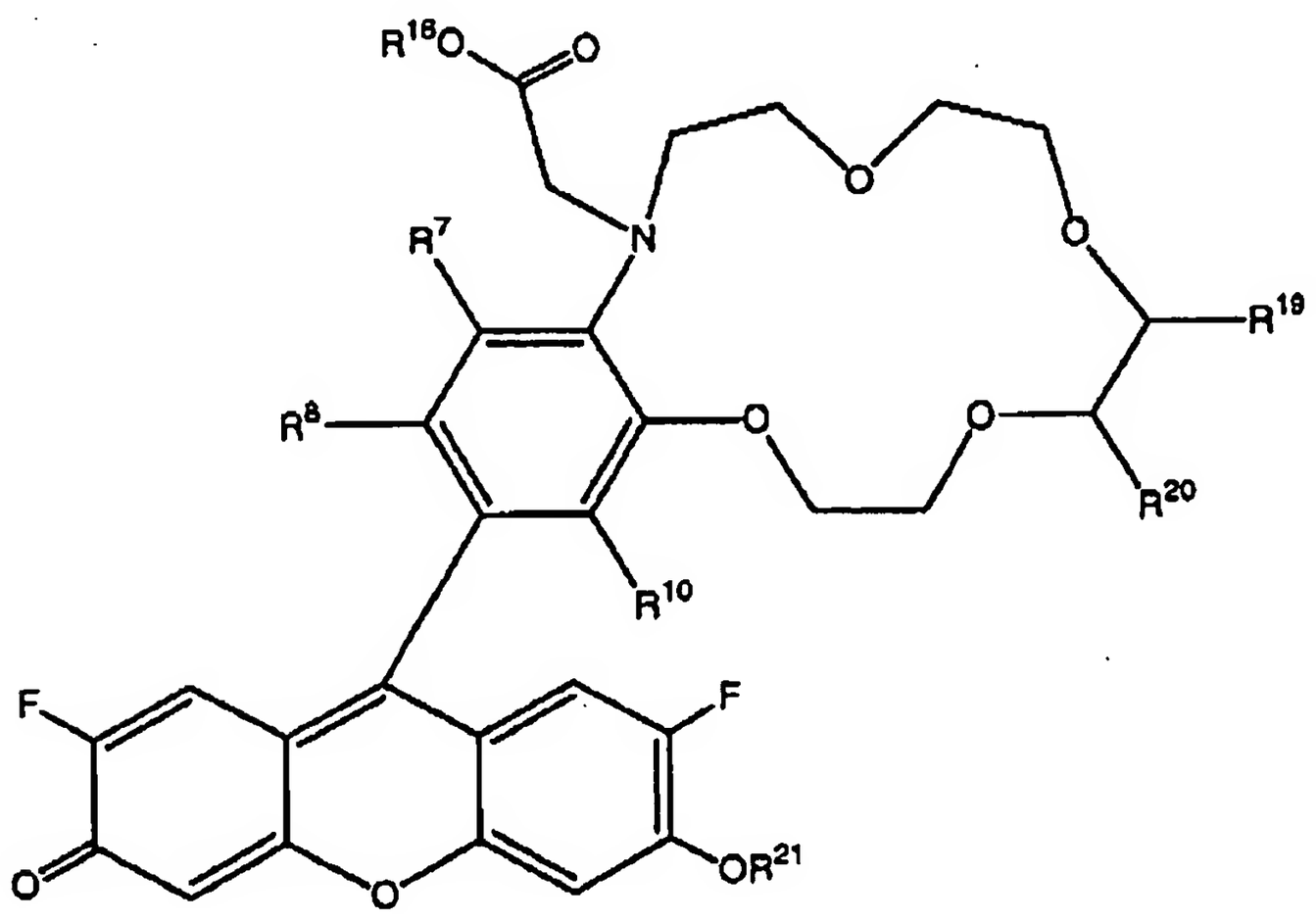
19. (Original) The compound according to Claim 18, wherein said R^1 is methyl or ethyl.

20. (Original) The compound according to Claim 19 wherein said R^{16} is selected from the group consisting of H, C_1-C_6 alkyl, benzyl, a biologically compatible esterifying group, and a biologically compatible salt.

21. (Original) The compound according to Claim 20 wherein said R^{16} is methyl.

22. (Original) The compound according to Claim 18 wherein said R^{17} and R^{18} are each methyl.

23. (Currently Amended) A compound having formula:



wherein R^{16} is selected from the group consisting of H, C_1-C_6 alkyl, benzyl, a biologically compatible esterifying group, and a biologically compatible salt;

R^{19} and R^{20} are selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl and C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16} and -(C=O)-$NR^{17}R^{18}$;

or R^{19} and R^{20} taken in combination form a fused six-membered benzo molety that is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

R^{15} is selected from the group consisting of H, C_1-C_6 alkyl, -L-R_X, -L-S_C and -L-DYE;

R^{16} is selected from the group consisting of H, C_1-C_6 alkyl, benzyl, a biologically compatible esterifying group, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE;

R^{17} and R^{18} are independently selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, alpha-acyloxyalkyl, trialkylsilyl, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;

each L is independently a covalent linkage;

each R_X is independently ~~a reactive group~~ an acrylamide, an activated ester of a carboxylic acid, a carboxylic ester, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an anhydride, an aniline, an amine, an aryl halide, an azide, an aziridine, a boronate, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a silyl halide, a sulfonyl halide, a thiol and a photoactivatable group;

each S_C is independently ~~a conjugated substance~~ an amino acid, a peptide, a protein, a polysaccharide, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell or a virus;

each DYE is independently a reporter molecule;

R^7, R^8, and R^{10} are independently selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl and C_1-C_6 alkoxy, each of which is optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or R^7 taken in combination with R^8 form a fused six-membered benzo moiety, which is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L--R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is optionally substituted by halogen, amino, hydroxy, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$; and,

R^{21} is selected from the group consisting of H, C_1-C_{18} alkyl, C_7-C_{18} arylalkyl and a lipophilic group each alkyl is optionally substituted by -(C=O)-R^{15}, -(C=O)-O-R^{16}, or C_1-C_6 alkoxy.

24. (Original) The compound according to Claim 23, wherein said R^7, R^8, and R^{10} are H.

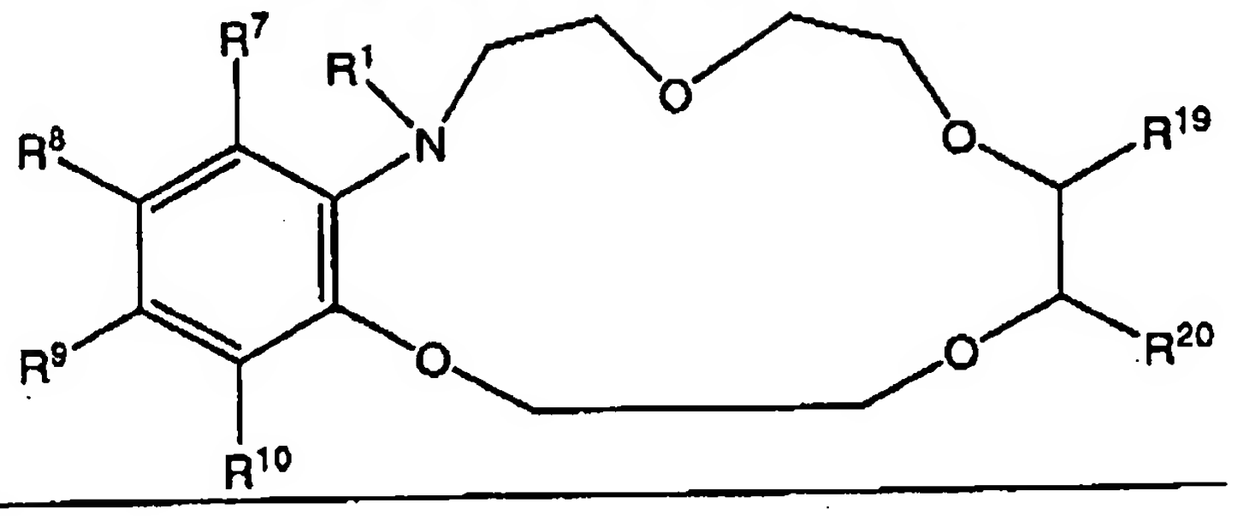
25. (Original) The compound according to Claim 24, wherein said R^{19} and R^{20} are H.

26. (Original) The compound according to Claim 25, wherein said R^{16} is methyl or a biologically compatible esterifying group.

27. (Original) The compound according to Claim 23 wherein said R^{16}, R^{19} or R^{20} is –L-Sc.

28. (Currently Amended) A composition comprising:

a) a compound ~~according to any one of Claims 1-27~~ having the formula



wherein

R^1 is selected from the group consisting of -L-R_X, -L-S_C, -L-DYE, C_1-C_{18} alkyl and C_7-C_{18} arylalkyl, each of which is optionally substituted by halogen, azido, nitro, nitroso, amino, hydroxy, cyano, C_1-C_8 alkoxy, an aryl or heteroaryl ring system, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, -(C=O)-$NR^{17}R^{18}$, C_1-C_8 alkylamino, C_2-C_{12} dialkylamino, C_1-C_8 alkyl or C_1-C_8 alkoxy, each of which is itself optionally substituted by halogen, amino (-$NR^{17}R^{18}$), hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16} or -(C=O)-$NR^{17}R^{18}$;

R^{15} is selected from the group consisting of H, C_1-C_6 alkyl, -L-R_X, -L-S_C and -L-DYE;

R^{16} is selected from the group consisting of H, C_1-C_6 alkyl, benzyl, a biologically compatible esterifying group, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE;

R^{17} and R^{18} are independently selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_8 carboxyalkyl, alpha-acyloxyalkyl, trialkylsilyl, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;

each L is independently a covalent linkage;

each DYE is independently a reporter molecule;

each Rx is independently an acrylamide, an activated ester of a carboxylic acid, a carboxylic ester, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an anhydride, an aniline, an amine, an aryl halide, an azide, an aziridine, a boronate, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a silyl halide, a sulfonyl halide, a thiol and a photoactivatable group;

each Sc is independently an amino acid, a peptide, a protein, a polysaccharide, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell or a virus;

R^{19} and R^{20} are independently selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl and C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or R^{19} and R^{20} taken in combination form a fused six-membered benzo moiety that is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

R^7, R^8, R^9 and R^{10} are independently selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy; -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{10}, taken in combination, form a fused six-membered benzo moiety, which is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is

optionally substituted by halogen, amino, hydroxy, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

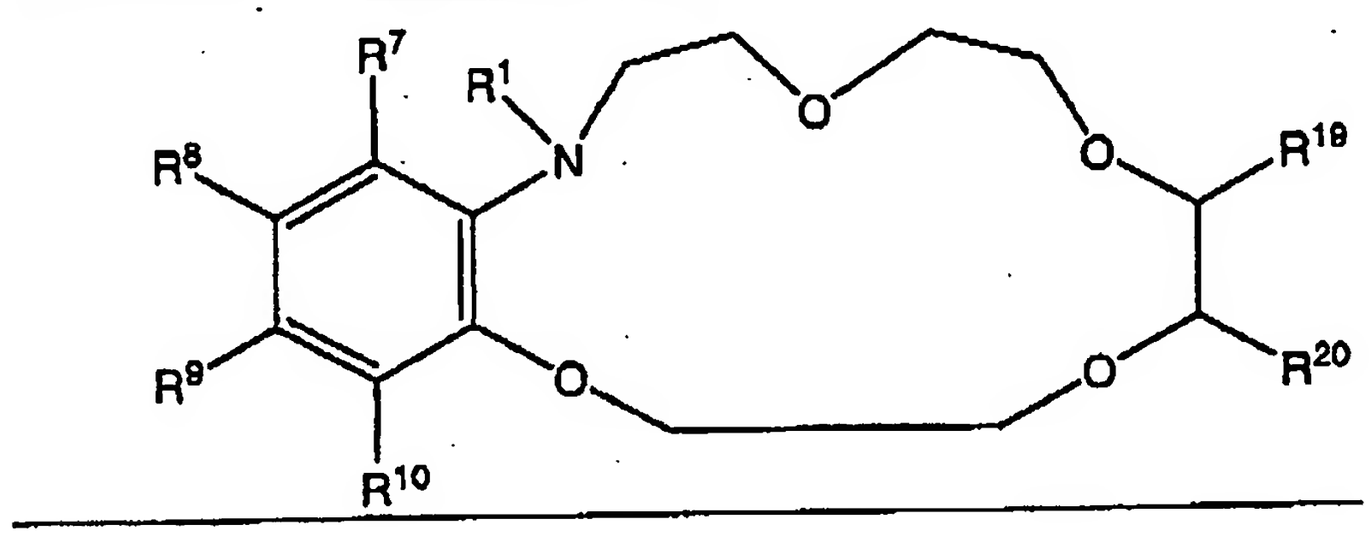
or any two adjacent substituents R^7-R^{10}, or R^{19} and R^{20}, taken in combination with each other, form a fused DYE; and,

b) a metal ion that is capable of being chelated by said compound.

29. (Original) The composition according to Claim 28, wherein said metal ion is selected from the group consisting of Na^+, Li^+, K^+, Ca^+, Zn^+ and Rb^+.

30. (Currently Amended) A method for binding a target metal ion in a sample, comprising steps of:

a) contacting said sample with a metal chelating compound ~~according to any one of Claims 1-27~~ having the formula



wherein

R^1 is selected from the group consisting of -L-R_X, -L-S_C, -L-DYE, C_1-C_{18} alkyl and C_7-C_{18} arylalkyl, each of which is optionally substituted by halogen, azido, nitro, nitroso, amino, hydroxy, cyano, C_1-C_8 alkoxy, an aryl or heteroaryl ring system, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, -(C=O)-$NR^{17}R^{18}$, C_1-C_8 alkylamino, C_2-C_{12} dialkylamino, C_1-C_8 alkyl or C_1-C_8 alkoxy, each of which is itself optionally substituted by halogen, amino (-$NR^{17}R^{18}$), hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16} or -(C=O)-$NR^{17}R^{18}$;

R^{15} is selected from the group consisting of H, C_1-C_8 alkyl, -L-R_X, -L-S_C and -L-DYE;

R^{16} is selected from the group consisting of H, C_1-C_8 alkyl, benzyl, a biologically compatible esterifying group, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE;

R^{17} and R^{18} are independently selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, alpha-acyloxyalkyl, trialkylsilyl, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;

each L is independently a covalent linkage;

each DYE is independently a reporter molecule;

each Rx is independently an acrylamide, an activated ester of a carboxylic acid, a carboxylic ester, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an anhydride, an aniline, an amine, an aryl halide, an azide, an aziridine, a boronate, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a silyl halide, a sulfonyl halide, a thiol and a photoactivatable group;

each Sc is independently an amino acid, a peptide, a protein, a polysaccharide, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell or a virus;

R^{19} and R^{20} are independently selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl and C_1-C_8 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or R^{19} and R^{20} taken in combination form a fused six-membered benzo moiety that is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

R^7, R^8, R^9 and R^{10} are independently selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{10}, taken in combination, form a fused six-membered benzo moiety, which is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is optionally substituted by halogen, amino, hydroxy, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{10}, or R^{19} and R^{20}, taken in combination with each other, form a fused DYE; and,

b) incubating said sample and said metal chelating compound for sufficient time to allow said compound to chelate said target metal ion whereby said metal ion is bound.

31. (Original) The method according to Claim 30, wherein said method further comprises illuminating said metal chelating compound with a suitable light source whereby said target ion is detected with the proviso that at least one of R^1, R^4, R^7, R^8, R^9, R^{10}, R^{19} or R^{20} is –L-DYE or at least two of R^7-R^{10} or R^{19} and R^{20}, taken in combination, form a fused DYE.

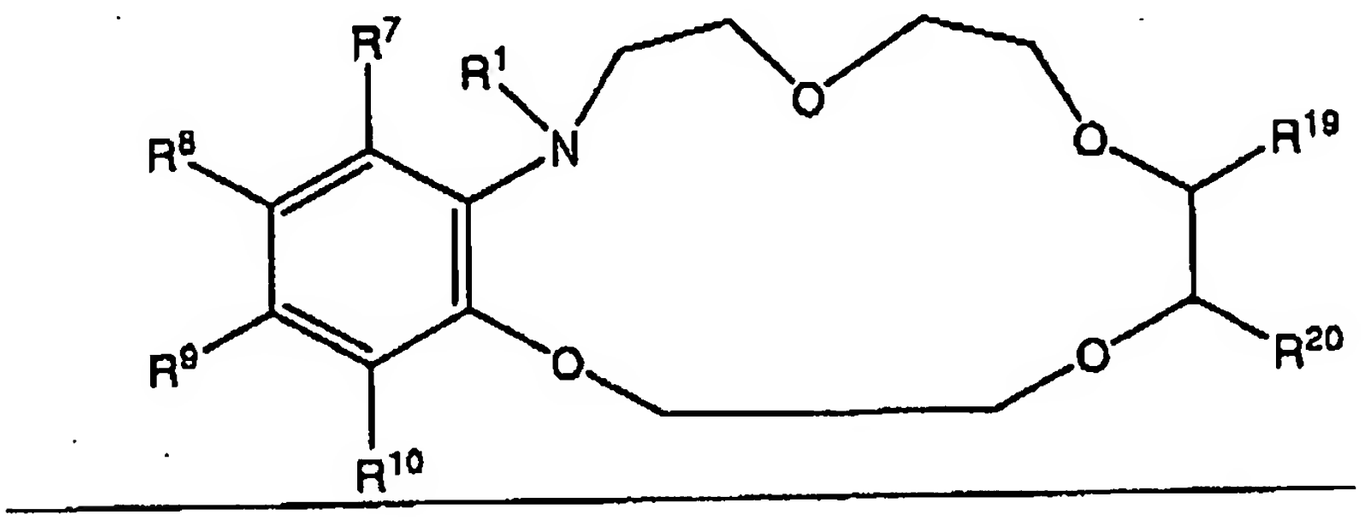
32. (Original) The method according to Claim 31, wherein said target metal ion is selected from the group consisting of Na^+, Li^+, K^+, Ca^+, Zn^+ and Rb^+.

33. (Original) The method according to Claim 32, wherein said target metal ion is Na^+.

34. (Original) The method according to Claim 32, wherein said sample comprises living cells, cellular components, proteins, peptides, buffer solutions or biological fluids.

35. (Currently Amended) A method for binding and detecting target ions in a live cell, said method comprises:

a) contacting a sample of live cells with a crown ether compound ~~according to any one of Claims 1-27~~ with the proviso that said compound comprise a DYE moiety and at least one lipohilic group, wherein the crown ether compound has the formula



wherein

R^1 is selected from the group consisting of -L-R_X, -L-S_C, -L-DYE, C_1-C_{18} alkyl and C_7-C_{18} arylalkyl, each of which is optionally substituted by halogen, azido, nitro, nitroso, amino, hydroxy, cyano, C_1-C_6 alkoxy, an aryl or heteroaryl ring system, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, -(C=O)-$NR^{17}R^{18}$, C_1-C_6 alkylamino, C_2-C_{12} dialkylamino, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino (-$NR^{17}R^{18}$), hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16} or -(C=O)-$NR^{17}R^{18}$;

R^{15} is selected from the group consisting of H, C_1-C_6 alkyl, -L-R_X, -L-S_C and -L-DYE;

R^{16} is selected from the group consisting of H, C_1-C_6 alkyl, benzyl, a biologically compatible esterifying group, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE;

R^{17} and R^{18} are independently selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, alpha-acyloxyalkyl, trialkylsilyl, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;

each L is independently a covalent linkage;

each DYE is independently a reporter molecule;

each Rx is independently an acrylamide, an activated ester of a carboxylic acid, a carboxylic ester, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an anhydride, an aniline, an amine, an aryl halide, an azide, an aziridine, a boronate, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a silyl halide, a sulfonyl halide, a thiol and a photoactivatable group;

each Sc is independently an amino acid, a peptide, a protein, a polysaccharide, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell or a virus;

R^{19} and R^{20} are independently selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl and C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or R^{19} and R^{20} taken in combination form a fused six-membered benzo moiety that is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

R^7, R^8, R^9 and R^{10} are independently selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{10}, taken in combination, form a fused six-membered benzo moiety, which is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is optionally substituted by halogen, amino, hydroxy, -(C=O)-R^{15}, -(C=O)-O-R^{15}, or -(C=O)-$NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{10}, or R^{19} and R^{20}, taken in combination with each other, form a fused DYE;

b) incubating said sample and said crown ether chelate compound for sufficient time to allow said compound to chelate said target metal ion; and,

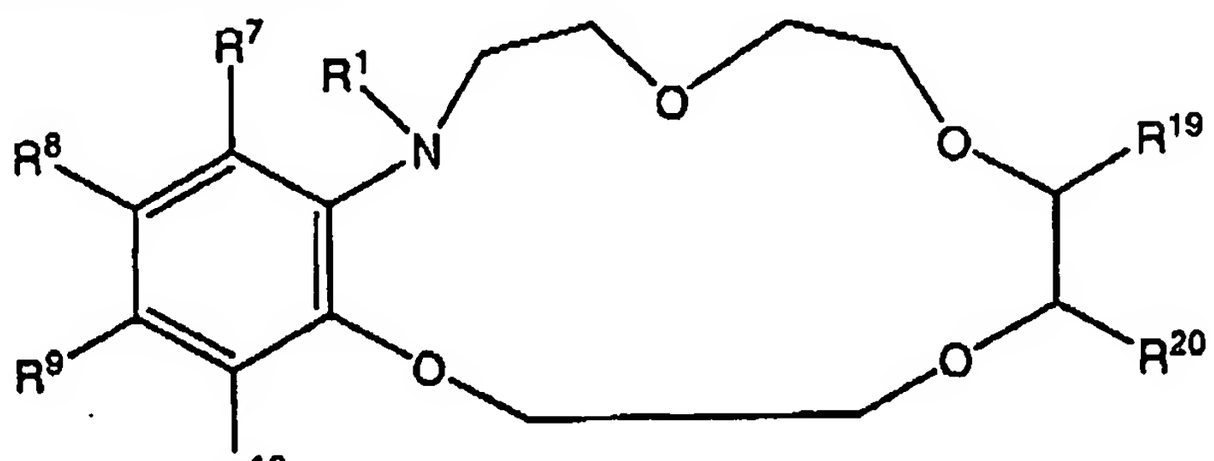
c) illuminate said sample with an appropriate wavelength whereby said target ion is detected in a live cell.

36. (Original) The method according to Claim 35, wherein said DYE moiety is substituted by a lipophilic group.

37. (Currently Amended) The ~~metha~~ method according to Claim 36 wherein said lipophilic group is an AM or acetate ester.

38. (Currently Amended) A kit for binding a metal ion in a sample, comprising:

a compound ~~according to any one of Claims 1-27~~ having the formula



wherein

R^1 is selected from the group consisting of -L-R_X, -L-S_C, -L-DYE, C_1-C_{18} alkyl and C_7-C_{18} arylalkyl, each of which is optionally substituted by halogen, azido, nitro, nitroso, amino, hydroxy, cyano, C_1-C_8 alkoxy, an aryl or heteroaryl ring system, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, -(C=O)-$NR^{17}R^{18}$, C_1-C_8 alkylamino, C_2-C_{12} dialkylamino, C_1-C_6 alkyl or C_1-C_8 alkoxy, each of which is itself optionally substituted by halogen, amino (-$NR^{17}R^{18}$), hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{16}, -(C=O)-O-R^{16} or -(C=O)-$NR^{17}R^{18}$;

R^{15} is selected from the group consisting of H, C_1-C_8 alkyl, -L-R_X, -L-S_C and -L-DYE;

R^{16} is selected from the group consisting of H, C_1-C_8 alkyl, benzyl, a biologically compatible esterifying group, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE;

R^{17} and R^{18} are independently selected from the group consisting of H, C_1-C_8 alkyl, C_1-C_8 carboxyalkyl, alpha-acyloxyalkyl, trialkylsilyl, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;

each L is independently a covalent linkage;

each DYE is independently a reporter molecule;

each Rx is independently an acrylamide, an activated ester of a carboxylic acid, a carboxylic ester, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an anhydride, an aniline, an amine, an aryl halide, an azide, an aziridine, a boronate, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a silyl halide, a sulfonyl halide, a thiol and a photoactivatable group;

each Sc is independently an amino acid, a peptide, a protein, a polysaccharide, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell or a virus;

R^{19} and R^{20} are independently selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_8 alkyl and C_1-C_8 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or R^{19} and R^{20} taken in combination form a fused six-membered benzo moiety that is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_8 alkyl or C_1-C_8 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

R^7, R^8, R^9 and R^{10} are independently selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_8 alkyl or C_1-C_8 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{10}, taken in combination, form a fused six-membered benzo moiety, which is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_8 alkyl or C_1-C_8 alkoxy, each of which is optionally substituted by halogen, amino, hydroxy, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{10}, or R^{19} and R^{20}, taken in combination with each other, form a fused DYE; and,

comprising one or more components selected from the group consisting of a calibration standard of a metal ion, an ionophore, a fluorescent standard, an aqueous buffer solution and an organic solvent.

CONCLUSION

In light of the amendments and remarks, reconsideration and withdrawal of the outstanding rejections is respectfully requested. All amendments are made in a good faith effort to advance the prosecution on the merits. Applicant respectfully submits that no amendments have been made to the pending claims for the purpose of overcoming any prior art rejections that would restrict the literal scope of the claims or equivalents thereof. Applicant reserves the right to subsequently take up prosecution of the claims originally filed in this application in continuation, continuation-in-part, and/or divisional applications.

It is submitted that this application is now ready for allowance. Early notice to this effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (541) 335-0203.

Respectfully submitted,

Date: April 5, 2006

Koren J. Anderson

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